LABORATORY

191 Urinalysis

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Definition

Urinalysis is the examination of urine for certain physical properties, solutes, cells, casts, crystals, organisms, or particulate matter. Because urinalysis is easy, cheap, and productive, it is recommended as part of the initial examination of all patients and should be repeated as clinically warranted. This chapter focuses on what the physician may do in a few minutes with a urine sample, reagent strips, a microscope, and an inquiring mind. Such analysis may lead to more sophisticated chemical, immunologic, or bacteriologic studies.

Technique

The rationale and technique of urinalysis are straightforward. Nevertheless, various circumstances may alter the information obtained. For example, one should not be surprised if the urine analyzed did not come from the patient named, or that the protein and red cells were added to the urine after it reached the collection bottle. Such illicit treatment of the sample is not frequent, but may be used in an attempt to justify disability, military discharge, or need for hospitalization. Interestingly, if the temperature of fresh urine is checked, it may help to diagnose factitious oral or rectal fevers.

A sample should be obtained that is free of skin epithelium or bacteria, glandular secretions, skin salves, hair, lint, talcum, or other debris. In rare circumstances, it may be necessary to have the sample passed under observation to assure its source and freedom from exogenously introduced materials.

Ordinarily, a suitable sample may be obtained from a male simply by asking that the foreskin, if present, be pulled back, that the initial part of the stream be allowed to pass into the toilet, that the next ounce or two be collected, and that the last part of the stream be discarded with the first. In females, care must be taken to separate the labia, and the urine is collected similarly. During menstrual flow, a tampon helps keep menstrual fluid from mixing with voided urine. The utility of cotton balls, soaps, and scrubs to cleanse the meatal area is dubious, as the initial 40 to 50 ml of urine flow that is discarded is generally adequate to flush away meatal debris. The sample should be examined while fresh—indeed, while still warm—to give best results. On standing, particulate matter sediments out, bacteria proliferate and alter pH, casts may dissolve, and crystals may be lost. Refrigeration may cause precipitation of orange red crystals of uric acid, which can be redissolved by rewarming the urine.

Approximately 10 ml of well-mixed urine is taken for microscopic examination. If the urine is alkaline, 1 ml of dilute acetic acid will help dissolve phosphates that may obscure formed elements. There is no "gold standard" for how fast or how long one should centrifuge urine. I have found 3000 rpm for 3 minutes to be convenient. Others have favored 2000 rpm for 5 minutes. After centrifugation, the supernatant is discarded; the sediment is resuspended in the few drops that remain. A drop of this is placed on a slide, covered with a coverslip, and examined microscopically. No stain is routinely needed, nor is phase contrast ordinarily required. The microscope is adjusted so that relatively low light is used, and the slide is scanned under low power (100×), high power (400×), and, when protein is present, polarizing light. Low-power examination serves to identify areas of interest, high power permits identification and quantification, and polarizing light aids in identification of doubly refractile fat bodies and certain crystals. Criteria for "positive" findings on microscopic examination have been established and are useful guides for review (Table 191.1).

While urine is being centrifuged, a macroscopic examination consisting of inspection and reagent strip testing should be done. The color and clarity of the urine are apparent on inspection. Causes of unusually colored urines are shown in Table 191.2. Turbidity of fresh urine suggests pus or red blood cells. When normal urine has cooled, crystals may precipitate and cause turbidity.

Available reagent-impregnated strips provide information regarding renal function, carbohydrate metabolism, acid–base balance, liver function, and bacteriuria. It is necessary to follow instructions closely to obtain reliable results. In certain circumstances, misleading information may be suggested to the unwary observer.

The performance characteristics of one reagent strip are shown in Table 191.3.

Basic Science

The glomerular and tubular physiology involved in the formation and delivery of urine to the bladder and the mech-

Table 191.1
Criteria for Classification of Positive Urinary Sediment Examinations Using Bright Field Microscopy

More than 5 erythrocytes, leukocytes, or renal tubular cells per high-power field (×430)
More than 3 hyaline casts, more than 1 granular cast, or presence of any other type of cast per low-power field (×100)
1+ (3 to 5) bacteria per high-power field (×430)
Presence of fungi, parasites, or viral inclusions
Presence of significant crystals (e.g., cystine) or a large number of crystals (e.g., uric acid)
Note: Finding formed elements equal or greater than those cited above proves an abnormality; finding fewer formed elements does *not* prove that no abnormality is present, especially if the urine is dilute (see Schumann and Greenberg, 1979; Szwed and Schaust, 1982).

Table 191.2
Colored Urine

Color	Cause or associated condition
Watery	Dilute urine
Yellow-orange	Normal urochrome pigments; pyridium therapy
Green	Pseudomonas infection; methylene blue or other blue dye; elevated urinary copper level; phenol; iodochlorhydroxyquin; amitriptyline hydrochloride; "chlorophyll" breath; fresheners
Brown	Bile, feces
Black	Melanoma; alkaptonuria; phenol (as a vehicle for an intravenous drug); black water fever; alphamethyldopa
Purplish	Porphobilin
Red	Hemoglobin; laxatives containing phenolphthalein; myoglobin; senna; beets; rifampin
Milky white	Pus; chyle; phosphates

anisms of bladder function are beyond the scope of this chapter.

The *specific gravity* of urine depends on a person's state of hydration, the integrity of the posterior pituitary, and the renal tubules. Normally, all urine leaving Henle's loop is dilute relative to plasma, and under forced hydration may contain as little as 50 mOsm/kg, roughly equivalent to a specific gravity of 1.001 or 1.002. Specific gravity of urine equals the weight of a given volume of urine divided by the weight of an equal volume of water:

$$SG = \frac{\text{Weight of urine}}{\text{Weight of water}}$$

When a urinometer is used, a correction must be made for temperature when very exact results are needed such that 0.001 is added or subtracted for each 3°C change above or below the calibration temperature recorded on the instrument. When protein is present in large amounts, all common methods are affected. There is no convenient correction factor for reagent strips. For refractometers or urinometers, it is necessary to subtract 0.003 for every 1 g/dl of protein in urine to be accurate. When glucose is present in large amounts, reagent strips should not be affected. It is necessary to subtract 0.004 from refractometer or urinometer readings for every 1 g/dl of glucose to correct the value. Values above 1.032 suggest the presence of exogenous solutes such as mannitol or iodinated contrast media.

Table 191.3
Reagent Strip Sensitivity for Specific Tests

Test	Normal range	Strip sensitivity[a]
Specific gravity	1.003 to 1.040	1.003 to 1.040
pH	5 to 9	5 to 9
Protein	<30 mg/dl	10 to 25 mg/dl albumin
Glucose	<100 mg/dl	100 mg/dl glucose
Ketone	Variable	5 to 10 mg/dl acetoacetic acid
Bilirubin	None	0.8 mg/dl
Blood	None	0.015 to 0.062 mg/dl hemoglobin
Nitrite	None	0.1 mg/dl nitrite ion

[a]Package Insert Revised 5/84. Ames Reagent Strip for Clini-Tek. Ames Division, Miles Laboratories.

The reagent strip method commonly available has three principal components: polymethylvinyl ether/maleic acid, bromothymol blue, and buffers. When specific gravity is high, the pKa of the polyelectrolyte is decreased and pH falls, resulting in a color change of the indicator. Highly buffered alkaline urine may, therefore, result in a factitiously low apparent specific gravity.

Urinary pH is an expression of the proton concentration in urine. Although the number of free protons excreted contributes only a trivial fraction of the approximately 80 mEq of acid an average person excretes daily, the free protons determine the efficacy of the titratable acid mechanism and the ammonium excretion mechanism, which together account for the bulk of excreted acid. When urinary pH is high, there are few proton acceptors in urine and the nonionic diffusion of NH_3 into the tubular lumen is relatively impeded. When pH is low, phosphates and other solutes efficiently accept protons and diffusion of NH_3 into the tubular lumen is facilitated. Normally, urine can be acidified to a pH value of 5.2 or less. Failure to do so in the face of systemic acidosis may indicate partial or complete renal tubular acidosis. Bacterial infection with urea-splitting organisms may produce an elevated urinary pH, so if fresh urine has leukocytes, bacteria, and an elevated pH, Proteus species would be suspected as the offending organism. (Other bacteria can split urea but are not as commonly responsible for urinary tract infection.)

Protein enters urine either because of altered glomerular permeability or because of tubular damage. Glomerular proteinuria always includes a large component of albumin. Tubular proteinuria is of low molecular weight, such as β_2-microglobulin. Reagent strip tests for protein are virtually (but not completely) specific for albumin, and depend upon the capacity of protein to change the color of an acid-base indicator at a constant pH maintained by a buffer in the strip section. Contrast media, tolbutamide, tolmetin, or penicillin, which may give false positive readings with heat and acetic acid, do not affect results with the reagent strip. Alkaline urine may give a false positive result with the reagent strip and a false negative result with acid precipitation techniques.

Glucose is normally present in human urine in small amounts. Glucose is usually not detectable because ketones, ascorbic acid, or other substances found in urine may cause false negative results by reagent strips even when urinary glucose approaches clearly abnormal values near 100 mg/dl. Copper reduction tests are not specific for glucose and may react with other hexoses, pentose, creatinine, uric acid, salicylates, and numerous other agents. When bacteria are present, glucose may be consumed, so a false negative could result from testing urine that is not fresh. When a hexokinase reagent strip is used, glucose concentrations below 2 mg/dl in morning urine from a fasting person correlate well with urinary tract infection. Large amounts of urinary glucose suggest diabetes mellitus, or, rarely, renal glycosuria.

Ketone bodies appear in urine as a consequence of accelerated fat metabolism. β-Hydroxybutyric acid is quantitatively greatest, followed by acetoacetic acid and acetone. When large amounts are present, a fruity odor may be detectable. The commonly available tests for ketone bodies depend on the development of a purple compound in the presence of nitroprusside and alkali. Such tests will react with acetone or acetoacetic acid but not with β-hydroxybutyric acid. L-Dopa will give a false positive result with the nitroprusside-based tests. A ferric chloride method is available that gives false positive results for both L-dopa and

salicylates. Ketone bodies are most likely to be present in the urine of an adult during diabetic ketoacidosis or when the patient has been fasting.

Bilirubin and *urobilinogen* appear in urine when there are abnormalities of bilirubin metabolism or liver function. Albumin-bound bilirubin (indirect bilirubin) is not water soluble and does not appear in the urine. Bilirubin conjugated with glucuronic or sulfuric acid is water soluble and appears in urine in amounts roughly correlated with the direct reacting serum bilirubin. The presence of conjugated bilirubin in detectable amounts (greater than 0.2 mg/dl) does not enable one confidently to distinguish between hepatocellular and obstructive jaundice, but does not commonly occur when hyperbilirubinemia is consequent to hemolysis. Reagent strips and tablets may give a false negative reaction when urine contains ascorbic acid. Phenothiazines may cause a false positive reaction in both cases.

When conjugated bilirubin reaches the bowel, bacterial action produces urobilinogen, which is reabsorbed into the portal circulation. Increased production of bilirubin or decreased hepatic clearance of urobilinogen from the portal circulation will increase the amount delivered to the kidney and excreted in the urine. Thus, hemolysis or hepatocellular dysfunction may increase urinary urobilinogen, while biliary obstruction will decrease delivery of conjugated bilirubin to the bowel, thereby reducing production of urobilinogen. Antibiotics, by altering bowel flora, may prevent production of urobilinogen.

These two tests, taken together, help to distinguish between hemolysis, hepatocellular disease, and biliary obstruction, as shown in Table 191.4.

Hemoglobin is not normally present in urine. It may appear if there is intravascular hemolysis so that hemoglobin is filtered into the urine, or if red cells break apart within the urinary tract, liberating hemoglobin. Myoglobin also causes reddish brown urine and reacts with the reagent strip for hemoglobin. Typically, if serum and urine are both red, hemolysis is more likely, as the molecular weight of hemoglobin impedes its filtration. If serum is normal in color and urine is red, myoglobin is more likely because its smaller size favors filtration. Alternatively, of course, clear serum and red urine may represent bleeding within the urinary tract itself.

False negative reagent strip results may occur if urine contains large amounts of ascorbic acid. False positive results may be seen if povidone iodine solutions are rinsed into urine before testing.

Clinical Significance

The reduction of nitrate to nitrite by bacteria with consequent color change in a solution or on a reagent strip is a useful indicator of bacteriuria, and when positive, should be taken seriously. A negative result gives *no* assurance that significant infection is not present. Urine may not have been retained in the bladder long enough for bacteria to have reduced nitrate, and this will invariably be the case when a urinary catheter is in place. Moreover, certain pathogens, such as *Streptococcus faecalis*, do not reduce nitrate at all.

Patients who have hypokalemia, hypercalcemia, protein malnutrition, or polydipsia will not be able to concentrate urine appropriately. Diuretics similarly impair concentrating ability, especially if kaliopenia develops. These conditions must be considered when interpreting specific gravity in the two circumstances for which it has greatest use: evaluation of possible acute renal failure, and evaluation of renal dysfunction to distinguish glomerular from tubulointerstitial disease. It is often said that a specific gravity of more than 1.020 makes acute renal failure less likely, but this is true only if the value is not spuriously elevated by endogenous or exogenous solutes. It is also said that specific gravity tends to be elevated in primary glomerular disease and reduced in tubulointerstitial disease, but this is of value only when factors that might elevate or depress specific gravity are not present.

Reagent strips cannot be relied on to detect globulins or light chains. The combination of a "negative" strip result and a positive acid-precipitation test would be suggestive of multiple myeloma. Those with an aging population in their practice may find it productive to use both methods.

Similarly, most reagent strips cannot be depended on to detect sugars other than glucose in urine. This may be a drawback in pediatric screening circumstances when inborn errors of metabolism may cause other sugars to appear in urine.

Remember that the presence of excess amounts of ascorbic acid in urine may affect three areas of the commonly used reagent strip: those that detect glucose, bilirubin, and hemoglobin. As people commonly take large amounts of ascorbic acid for various reasons, it is prudent to inquire specifically about this before interpreting the reagent strip results.

Careful analysis of the sediment usually takes about 5 minutes of patient examination of the area under the coverslip. The principal elements to be identified and quantified are cells, casts, and crystals.

Cells are ordinarily divided into red blood cells, white blood cells, epithelial cells, and atypical cells.

Red blood cells are associated with primary parenchymal disease such as glomerulonephritis, diabetes mellitus, polycystic kidney disease, drug reactions (e.g., penicillin), or collagen vascular disease. They may also be found with renal calculi, tumors of the urinary tract, upper or lower tract infections (cystitis or prostatitis), and trauma. The differential diagnosis of hematuria is considered in some detail in Chapter 184.

Table 191.4
Profiles of Urine Urobilinogen and Bilirubin in Health and Disease

	Health	Hemolysis	Hepatic disease	Biliary obstruction
Urine urobilinogen	Normal	Increased	Increased	Low or absent
Urine bilirubin	Negative	Negative	Positive or negative	Positive

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White blood cells suggest inflammatory processes, with infection being the commonest of these. Other inflammatory stimuli, such as collagen vascular disease and allergic interstitial nephritis, also may cause pyuria. There is practical value in evaluating the pyuria by the company it keeps; for example, if white cells are found with red cells and red cell casts, then collagen vascular disease is more likely. If white cells are found by themselves in a patient with an acute onset of fever and dysuria, then infection is more likely.

Epithelial cells from any site in the urinary tract may be found in the sediment. Drugs, toxins, ischemia, instrumentation of the urinary tract, and tumors are among the causes for such cells to be sloughed into the sediment. Urine cytology studies may have particular utility when large numbers or unusual forms of epithelial cells are seen.

Casts are formed within the renal tubules and have a matrix of translucent protein that, by itself, forms the substance of the so-called hyaline cast. When there are inclusions in the cast such as red cells or white cells, they permit conclusion that the cells originated within the kidney. This is especially important when there is consideration of vasculitis or of pyelonephritis. Degenerating cellular debris may form granular casts. Hemoglobin, myoglobin, or bilirubin may be incorporated into pigmented casts.

Lipiduria is an important finding because of its association with the nephrotic syndrome. Fat may appear in urine as free fat, as inclusions within an oval fat body, or as the characteristically doubly birefringent cholesterol ester that is referred to as a "Maltese cross" after the cruciform emblem emblazoned on the shields and tunics of the Knights of Malta. Lipiduria is so abnormal that when found, it strongly suggests either fat embolization of the kidneys or the nephrotic syndrome, and the clinical context will readily differentiate in most cases. Finding lipiduria in a patient with proteinuria and edema permits one to anticipate the diagnosis of nephrotic syndrome while waiting for the laboratory to quantify cholesterol and serum albumin.

It is useful to look for bacteria. If the urine is truly fresh, the presence of even one bacterium per oil immersion field of unspun, gram-stained urine correlates reasonably well with a colony count of greater than 100,000 colonies per ml (Wilson, 1975).

There has been controversy in recent years as to whether microscopic urinalysis is always necessary, especially if the macroscopic urinalysis is entirely negative. In the majority of cases, it will prove that when specific gravity is over 1.020, and the macroscopic examination is completely normal, the microscopic examination will likewise be normal. There will also be false negative results, and it is estimated that these will vary from 3 to 37% of all cases (Schumann and Greenberg, 1979; Szwed and Schaust, 1982). Even when a special type of reagent strip is used, which is sensitive to leukocyte esterase, up to 3.3% of positive findings may be missed (Shaw, Poon, and Wong, 1985). With routine reagent strips, the same authors found a 13% false negative result rate. Certainly, one could not delete the microscopic examination in a patient with signs or symptoms of hypertension or renal disease. In other cases, if it is omitted, one must accept missing 10% or more of positive findings—a loophole too large for most physicians' comfort.

Certain circumstances may warrant special stains as an adjunct to routine urinalysis. It has been suggested that a Wright's stain of the sediment may help to distinguish glomerular from lower tract sources of hematuria (Chang, 1984). Technically, it is useful to add a drop of albumin solution to the sediment to obtain clear slides after staining. Glomerular lesions produce dysmorphic RBCs, while distal lesions produce cells similar to those seen in peripheral blood. Such stains would have to be done on fresh urine, as hypotonic urine would lead to cell lysis, and hypertonic urine to pyknotic cells if the urine stood for any length of time. The same staining technique may be used to search for eosinophiluria when allergic interstitial nephritis is suspected. This may be helpful when B-lactam antibiotics, nonsteroidal inflammatory drugs, or other potentially toxic agents are used. Quantification of eosinophils as a percentage of total urinary white blood cells is helpful. When over 5% of urinary white cells are eosinophils, interstitial nephritis is more likely. When less than 5% are eosinophils, infection is commonly the cause (Corwin, Korbat, and Schwartz, 1985).

The urine sediment may also reveal crystals, parasites, foreign bodies, spermatozoa or other findings. Assessment of the meaning of such findings—indeed, assessment of all the findings of urinalysis—depends upon the inquiring mind that asks an appropriate question. The mind, therefore, is the most important element of informative urinalysis. Because of that, it is best for the physician rather than anyone else to review the macroscopic findings, inspect the sediment, and interpret results for that unique patient, even as the physician does the physical examination rather than depend upon a technician's report.

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